6 - DNA Replication

When James Watson and Francis Crick determined the structure of the DNA double helix, they noticed that the structure of DNA provided clues to how DNA is copied prior to cell division. This copying process is called **DNA replication** (see **figure 6.1**).

Overview of DNA Replication

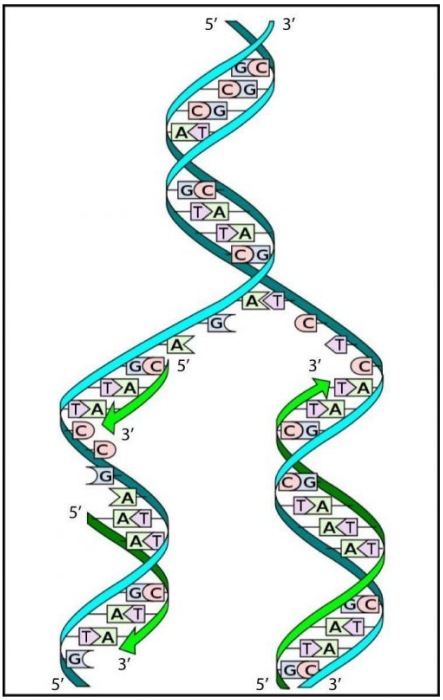


Figure 6.1 ***Overview of DNA Replication*** *--- Image created by SL*

During DNA replication, the two original DNA strands within the double helix separate, and two new strands of DNA are synthesized. The two original strands are called **template DNA** or **parental DNA strands**; each of the newly synthesized DNA strands is called a **daughter DNA strand**.

When DNA nucleotides (deoxyribonucleoside triphosphates or **dNTPs**) are used to generate the daughter DNA strands, the AT/GC rule is followed. Hydrogen bonds are formed between the nitrogenous bases within the incoming nucleotides and the template strand bases. Then a phosphodiester bond is formed between the free 5’ phosphate on the incoming nucleotide and the free 3’ hydroxyl group on the growing daughter DNA strand.

The dNTPs used as the substrates for DNA synthesis include **deoxyadenosine triphosphate (dATP)**, **deoxythymidine triphosphate (dTTP)**, **deoxycytidine triphosphate (dCTP)**, and **deoxyguanosine triphosphate (dGTP)**.

Key Questions

- What is a template DNA strand?
- What is a daughter DNA strand?
- What are dNTPs?

A. DNA Replication in Bacteria

Origin of Replication in Bacteria

The site on the bacterial chromosome where DNA replication begins is the **origin of replication** (see **figure 6.2**). The bacterium *E. coli* has a single origin of replication called ***OriC***. *OriC* is a 275 base pair (bp)-long region that contains important DNA sequences, such as:

- **AT-rich sequences**. There are three repeats of the **AT-rich sequences** in *OriC*. These AT-rich sequences are significant as only two hydrogen bonds hold AT base pairs together in DNA. As a result, less energy is required to separate AT base pairs than GC base pairs. The DNA strand separation that is required during DNA replication initiates at these AT-rich sequences.
- **DnaA box sequences**. There are five DnaA protein (see below) binding sites within *OriC*. These DnaA binding sites are called **DnaA boxes**.
- **GATC methylation sequences**. There are approximately twelve **GATC methylation sequences** within *OriC*. Methylation of the adenine bases within each GATC methylation sequence serves as an activation signal for DNA replication.

DNA replication begins at *OriC* and proceeds in both directions (clockwise and counterclockwise) along the circular bacterial chromosome (**bidirectional replication**). A **replicon** is the DNA replicated from a single origin. Since the entire *E. coli* chromosome is replicated from a single origin, the entire chromosome is one replicon**.**

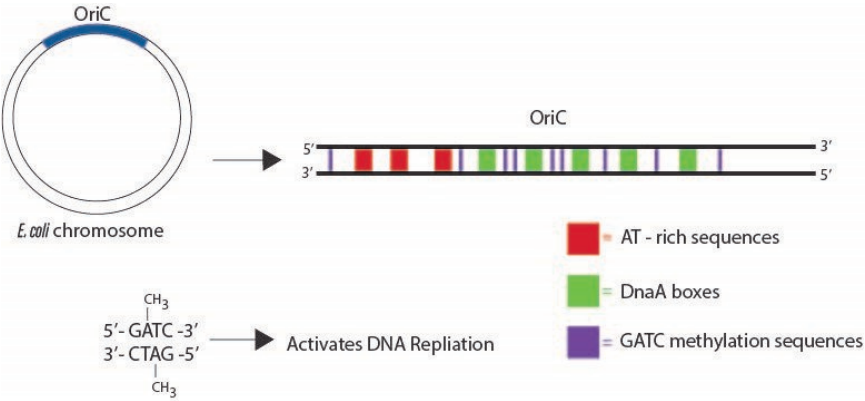


Figure 6.2 ***OriC in E.coli ---*** *Image created by KMD*

Key Questions

- What are the names of the three types of sequences found in *OriC*?
- What are the functions of these three types of sequences?
- What is a replicon?

Replication Initiation

When a bacterial chromosome initiates DNA replication, the following process occurs (see **figure 6.3**):

1. **DnaA proteins bind to the DnaA box sequences**. **DnaA** proteins bind to and later cleave ATP. When DnaA is bound to ATP, DnaA binds tightly to the DnaA box sequences within *OriC*.

2. **The origin forms a loop and the individual DNA strands separate.** When DnaA proteins (with associated ATP) are bound to the DnaA box sequences, DnaA proteins can then bind to each other to form a complex, form a loop in the DNA, and promote DNA strand separation within the AT-rich sequences. This looping of the DNA and strand separation requires ATP cleavage by DnaA. After ATP is cleaved, DnaA is released from *OriC*.
3. **DNA helicases** (also called **DnaB** proteins) **bind to the separated DNA strands.**
4. **DNA helicases continue to separate the DNA strands in both directions forming two replication forks**. The separation of DNA strands by DNA helicase occurs by breaking the hydrogen bonds holding the two DNA strands together. Template strand separation starts in *OriC* and continues beyond the origin, moving in both directions along the circular bacterial chromosome. DNA helicase binds and cleaves ATP to catalyze DNA strand separation.
5. **Single-stranded DNA binding proteins (SSBPs) bind to the single-stranded DNA.** SSBPs prevent the DNA strands, separated by DNA helicase, from reforming hydrogen bonds, so that DNA replication can proceed.

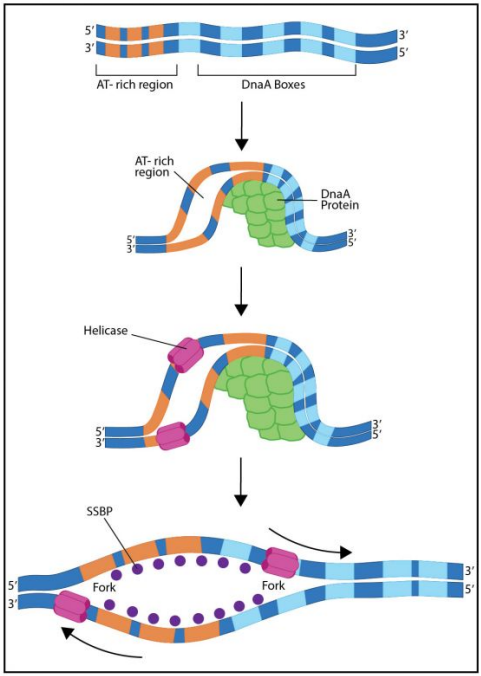


Figure 6.3 ***Replication Initiation in Bacteria*** *--- Image created by SL*

Coordinating Replication with Cell Division

Some bacteria can divide very quickly. For example, the cell division time of *E. coli* is approximately 20 minutes. If DNA replication does not keep up with the division of the cytoplasm, daughter cells will be formed that lack chromosomes. On the other hand, if DNA replication occurs too quickly, daughter cells may contain more than one copy of the chromosome.

How is DNA replication and division of the bacterial cytoplasm coordinated? *E. coli* cells coordinate these two processes by regulating the initiation of DNA replication. There are two general

ways that DNA replication is coordinated with cell division in *E. coli*:

- **Limiting the amount of active DnaA protein**. To initiate DNA replication, DnaA proteins must be bound to all five DnaA box sequences within *OriC*. When a bacterial cell decides to replicate the DNA, there is only enough active DnaA proteins in the cell to bind to the five DnaA boxes within a single copy of *OriC*. After DNA replication occurs, there are now two copies of *OriC* in the same cell. At this point, there is not enough active DnaA protein present to start a second round of DNA replication. By the time additional copies of the DnaA proteins are synthesized, the cell has divided its cytoplasm producing two daughter cells.
- **GATC methylation**. An enzyme called **DNA adenine methyltransferase (Dam)** recognizes the GATC methylation sequences in *OriC* and methylates the adenine, forming methyladenine (A^{me}). Recall that there are numerous GATC methylation sequences in *OriC*. Prior to replication, Dam ensures that the adenines in all GATC sequences are methylated. If these GATC sites are methylated, DNA replication is initiated. After DNA replication, two DNA molecules are found in the same cell. Within each of these two molecules, the parental DNA strands contain methylated adenine, but the daughter DNA strands do not. A new round of DNA replication does not occur until Dam methylates the adenines within the daughter DNA strands (this can take several minutes). Thus, an *E. coli* cell has enough time to divide its cytoplasm prior to initiating a second round of DNA replication.

Key Questions

- What are the names of four proteins involved in the initiation step of replication in *coli*?
- What are the functions of these four proteins?

Replication Elongation

The elongation stage of DNA replication in bacteria consists of the following steps (see **figure 6.4**):

1. **RNA primers are synthesized**. After the parental DNA strands have separated, small RNA molecules (10-12 nucleotides long) are synthesized that are complementary to the template DNA strands. These RNA **primers** provide the free 3’-OH groups required by DNA polymerases to initiate DNA synthesis.
2. **DNA synthesis occurs using the parental DNA strands as templates.** One rule of DNA replication is that the daughter DNA strands are synthesized in the 5’ to 3’ direction. However, because the parental DNA strands are antiparallel to the daughter DNA strands, DNA polymerases read the parental DNA strands in the 3’ to 5’ direction as the daughter DNA strands are synthesized. Since DNA polymerases only synthesize the daughter DNA strands in the 5’ to 3’ direction, the two DNA strands synthesized at each replication fork are synthesized in opposite directions. One newly synthesized DNA strand is called the **leading strand.** The leading strand is synthesized in the same direction that the replication fork is separating the daughter DNA strands. The leading strand uses only one RNA primer and DNA synthesis is **continuous**. The other newly synthesized DNA strand at the replication fork is called the **lagging strand.** The lagging strand is synthesized as a series of **Okazaki fragments** (1000–2000 nucleotides long in

bacteria) in the opposite direction the replication fork is separating the daughter DNA strands. Each Okazaki fragment has an RNA primer, and the lagging strand is synthesized in a **discontinuous** manner.

3. **The RNA primers are removed.** Removing the RNA primers results in a gap between each Okazaki fragment.
4. **DNA synthesis fills the gaps left by the RNA primers.**
5. **The adjacent Okazaki fragments are linked together.** Ligation of the adjacent Okazaki fragments forms a continuous lagging strand.

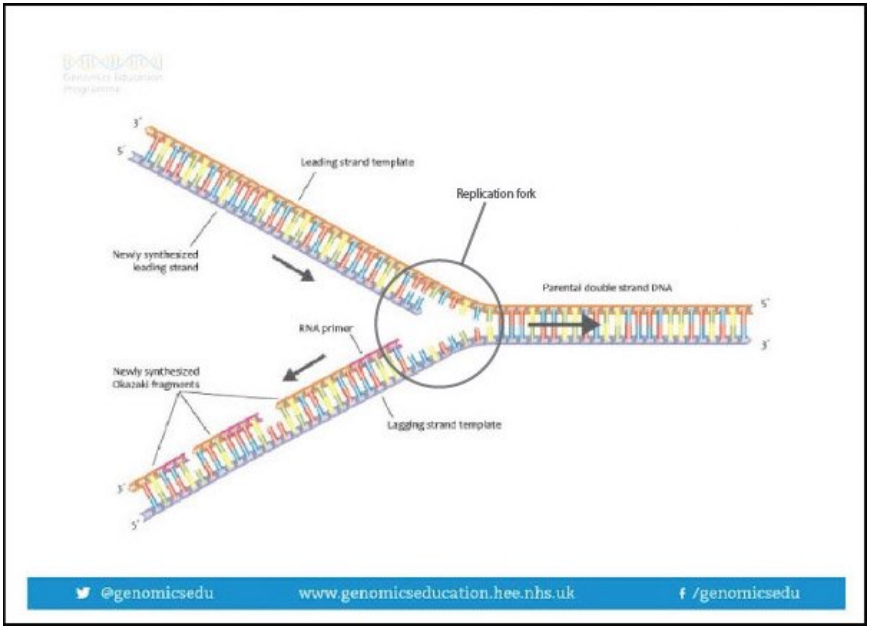


Figure 6.4 ***Replication Elongation ----*** *Image by Genomics Education Programme. Image licensed under CC BY 2.0*

Key Questions

- What are the major events that occur in the elongation stage of DNA replication in bacteria?
- What is the direction of DNA replication?
- What is the difference between the leading and lagging DNA strands?

Proteins Involved in Elongation

The following proteins are involved in the elongation stage of DNA replication in bacteria (see **figure 6.5**):

- **DNA helicase**. DNA helicase continues to separate the two parental DNA strands as the replication forks proceed from *OriC* clockwise and counterclockwise around the circular *E. coli* chromosome. DNA helicase consumes ATP as the replication forks proceed around the chromosome.
- **Single-stranded DNA binding proteins (SSBPs).** SSBPs prevent the template DNA strands, separated by DNA helicase, from reforming hydrogen bonds.
- **DNA gyrase.** The separation of the parental DNA strands by DNA helicase produces twisting called **positive supercoiling** ahead of each replication fork. This positive supercoiling can be lethal to a bacterial cell if left unchecked. DNA gyrase functions to relieve this positive supercoiling by introducing **negative supercoils** ahead of each replication fork. DNA gyrase consumes ATP as it forms negative supercoils.
- **DNA primase.** To synthesize the daughter DNA strands, a short sequence (10–12 nucleotides) of RNA called a **primer** is synthesized by DNA primase. The **leading strand** (DNA synthesis in the same direction as the movement of the replication fork) requires only a single RNA primer, while the **lagging strand** (DNA synthesis in the opposite direction as the

movement of the replication fork) requires many RNA primers. Since DNA primase synthesizes an RNA sequence, DNA primase consumes the RNA nucleotides ATP, UTP, CTP, and GTP as primers are made.

- **The DNA polymerase III holoenzyme.** The DNA polymerase III holoenzyme synthesizes the daughter DNA strands (both the leading and lagging strands) in the 5’ to 3’ direction. A single DNA polymerase III holoenzyme synthesizes both the leading and lagging DNA strands at each replication fork simultaneously (see below). The DNA polymerase III holoenzyme synthesizes the leading and lagging strands by consuming the nucleotides dATP, dTTP, dCTP, and dGTP.
- **DNA polymerase I.** DNA polymerase I removes the RNA primers and synthesizes DNA to fill in the gaps left by the removed RNA primers. DNA synthesis by DNA polymerase I also occurs in the 5’ to 3’ direction. DNA polymerase I consumes the nucleotides dATP, dTTP, dCTP, and dGTP as it synthesizes DNA.
- **DNA ligase.** DNA ligase forms the final covalent bond that links the adjacent Okazaki fragments into a continuous daughter strand. DNA ligase consumes ATP.

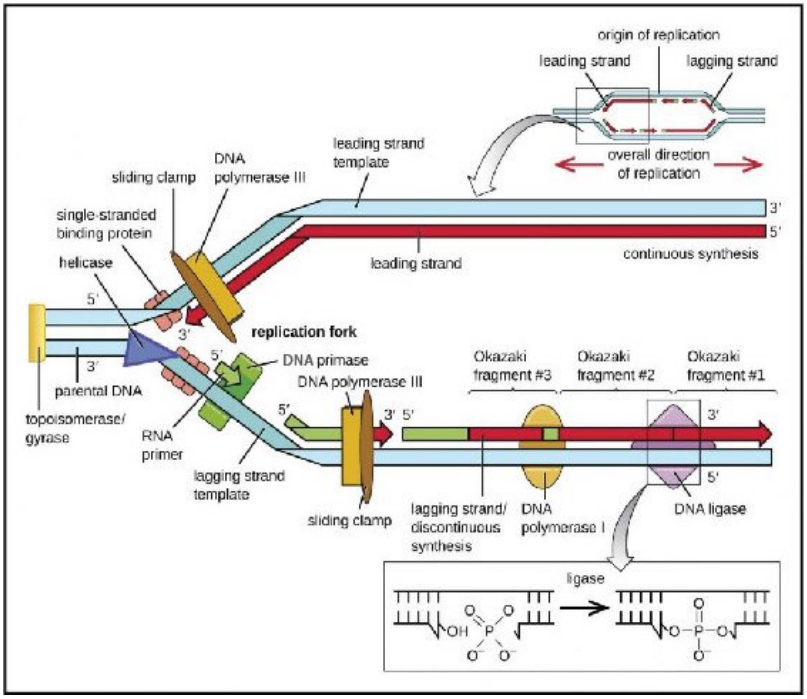


Figure 6.5 ***Bacterial Replication Proteins*** *--- This image is used from OpenStax (access for free at https://books.byui.edu/-vuzA)*

Key Questions

- What are the functions of the seven proteins involved in replication elongation in *E. coli*?
- List four replication elongation proteins that consume ATP.
- List two replication elongation proteins that consume dNTPs.

DNA Polymerase III Holoenzyme

DNA polymerase III is a **holoenzyme** (multi-protein enzyme complex) composed of at least ten unique protein types (see **figure 6.6**). Each of these unique protein types within the DNA polymerase III holoenzyme is present in multiple copies, making the overall

composition of the holoenzyme quite complex. The protein subunit composition of the DNA polymerase III holoenzyme is as follows:

- **Two alpha (α) protein subunits.** The α protein subunits of the DNA polymerase III holoenzyme carry out the **5′ to 3′ polymerase** activity (DNA synthesis activity). One α subunit synthesizes the leading strand; the other α subunit synthesizes the lagging strand.
- **Four beta (β) protein subunits.** The β protein subunits form sliding clamps that attach the two α subunits to the two template DNA strands. These β subunits slide along with the template DNA strands during DNA replication, preventing the α subunits from falling off during replication.
- **Two epsilon (ε) protein subunits.** The ε protein subunits of DNA polymerase III possess **proofreading** activity (see below) that fixes mistakes made during DNA replication.
- **Accessory protein subunits.** These accessory protein subunits load the α and β subunits onto the RNA primers during lagging strand synthesis and maintain the stability of the DNA polymerase III holoenzyme.

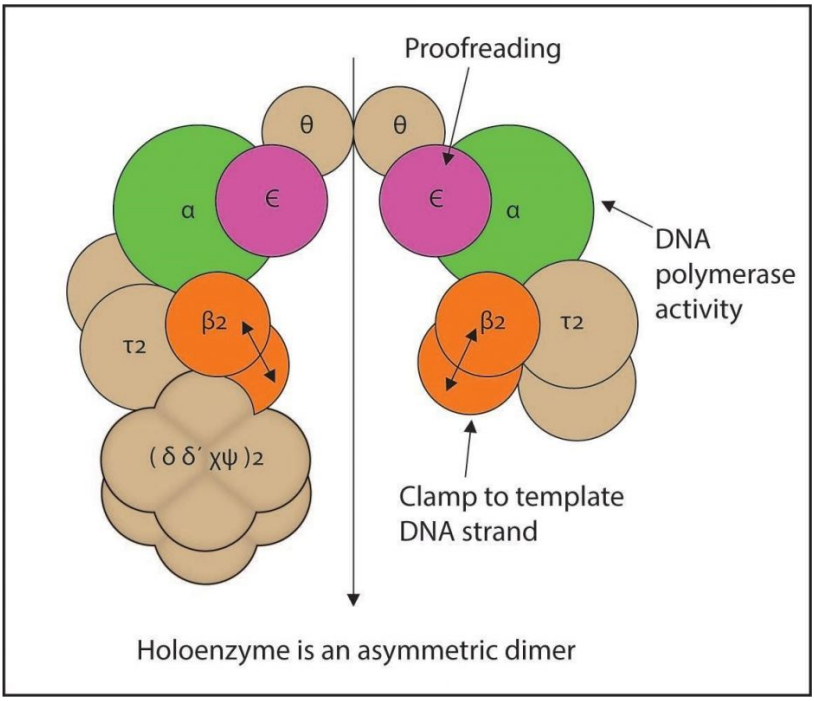


Figure 6.6 ***DNA Polymerase III Holoenzyme*** *--- Image created by SL*

Key Questions

- What are the functions of the α, β, and ε subunits of the DNA polymerase III holoenzyme?

DNA Replication Proteins form Complexes

Many of the DNA replication enzymes described above are not actually separate entities. Each enzyme has a very distinct function; however, several of these enzymes are physically linked to each other to form multiprotein "machines." For example, the **primosome** is a protein complex formed by the association of DNA helicase and DNA primase. The primosome moves along the DNA separating the DNA

strands and simultaneously synthesizing lagging strand RNA primers.

Moreover, the primosome itself is part of a larger multi-subunit complex called the **replisome**. The replisome includes the following:

- The primosome components (DNA helicase, DNA primase).
- A DNA polymerase III holoenzyme (including the α, β, ε, and accessory protein subunits).

There is a single replisome per replication fork in the bacterium *E. coli*. Since a replicating bacterial chromosome has two replication forks, there are two replisomes per bacterial cell.

For the leading and lagging DNA strands to be synthesized by the same replisome, the lagging strand forms a single-stranded loop that extends from the replisome complex. After synthesis of an Okazaki fragment, the DNA polymerase III α subunit that is synthesizing the lagging strand releases from the template DNA and binds to an RNA primer nearer to the replication fork.

Key Questions

- What are the protein components of the primosome?
- What are the protein components of the replisome?

Other DNA Polymerases in Bacteria

In the bacterium *E. coli*, there are five DNA polymerases. We will focus our attention on DNA polymerases I and III, as these two enzymes are involved in DNA replication. The other DNA polymerases (DNA polymerase II, IV, and V) are involved in repairing bacterial DNA that has been damaged by environmental agents.

DNA polymerase III (also called the DNA polymerase III holoenzyme; see above) replicates most of the DNA (has **5’ to 3’**

polymerase activity). DNA polymerase III synthesizes the leading and lagging strands and contains **3’ to 5’ exonuclease** activity (proofreading activity; see below).

DNA polymerase I is composed of a single protein subunit and functions to remove RNA primers using a **5’ to 3’ exonuclease** activity. DNA polymerase I also fills in the gaps left by the removed RNA primers with DNA via its **5’ to 3’ polymerase** activity and has **3’ to 5’ exonuclease activity** (proofreading activity; see below).

DNA polymerases have some unique features: DNA polymerases require a free 3’-OH group provided by the primer to begin DNA synthesis. The primer used within cells is RNA; however, DNA polymerases can use DNA primers to synthesize DNA as well. In fact, DNA primers are commonly used when synthesizing DNA in the lab (see Part 8). Also, DNA polymerases synthesize the growing daughter strand in the 5’ to 3’ direction only.

Key Questions

- What are the names and functions of the two enzymatic activities of the DNA polymerase III holoenzyme?
- What are the names and functions of the three enzymatic activities of DNA polymerase I?
- What are two unique features of all DNA polymerases?

DNA Polymerase Mechanism

DNA polymerases use the chemical energy stored within the high energy phosphate bonds of deoxyribonucleoside triphosphate (**dNTP**) molecules to synthesize the growing daughter DNA strand. The DNA polymerase mechanism is as follows (see **figure 6.7**):

1. The DNA polymerase reads a nitrogenous base in the template DNA strand and binds to the complementary dNTP according to

the AT/GC rule. The incoming dNTP forms hydrogen bonds with the complementary base in the template DNA strand.
2. The free 3′-OH group on the growing daughter DNA strand reacts with the free 5′ phosphate group on the incoming dNTP.
3. A high energy bond within the dNTP is broken releasing two of the phosphate groups in the form of a chemical called **pyrophosphate (PP_i)**.
4. The released energy is used to synthesize a new phosphodiester bond between the 3′ end of the growing DNA strand and the 5′ end of the incoming nucleotide.

The DNA polymerase III holoenzyme is **processive**. Processivity means that the DNA polymerase III holoenzyme can add many nucleotides to the growing DNA strand without falling off the template DNA strand. This processivity is due to the β subunits (sliding clamps) found within the DNA polymerase III holoenzyme.

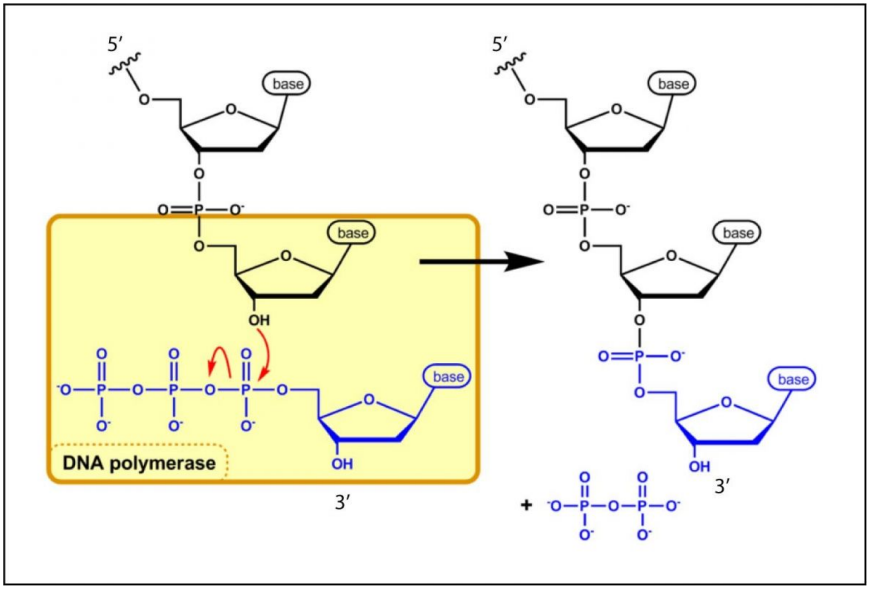


Figure 6.7 ***DNA Polymerase Mechanism*** *--- Image created by Michal Sobkowski and is licensed under CC BY 3.0.*

Key Questions

- Describe the DNA polymerase mechanism.
- What is meant by the phrase, “DNA polymerases are processive?”

Proofreading by DNA Polymerases

Wrong nucleotides (i.e., nucleotides that form base pairs that do not follow the AT/GC rule) are incorporated into the daughter DNA strand very rarely. For example, the DNA polymerase III holoenzyme is thought to make a mistake once every 10–100 million nucleotides incorporated into a DNA strand. This accuracy during DNA synthesis is called **fidelity**; DNA polymerase I and the DNA polymerase III holoenzyme are said to have high fidelity. The fidelity of DNA polymerases is the result of:

- **The stability of the hydrogen bonds between AT and GC.** Mismatched base pairs fail to form hydrogen bonds altogether or result in less stable hydrogen bonds.
- **The active site of DNA polymerases is very specific.** A covalent bond fails to form between the free 3’-OH group of the growing DNA strand and the free 5’ phosphate group of the incoming dNTP unless correct base pairing occurs.
- If an incorrect base pair is accidently formed, the DNA polymerase can pause, recognize the mismatch, and remove it (see **figure 6.8**). This **proofreading activity** is called the **3’ to 5’ exonuclease activity** of the enzyme. Once proofreading is complete, the DNA polymerase can continue incorporating dNTPs into the growing daughter DNA strand.

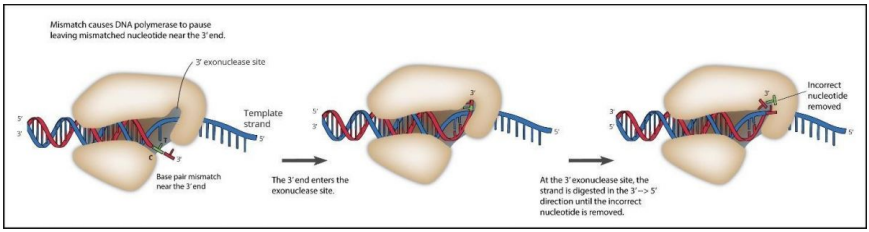


Figure 6.8 ***Proofreading*** *--- Image created by SL*

Key Questions

- What is meant by proofreading?
- Which enzymatic activity, found in DNA polymerase I and the DNA polymerase III holoenzyme, is responsible for proofreading?
- What is meant by the phrase, “DNA polymerases display high fidelity?”

Termination of Replication in Bacteria

DNA replication in *E. coli* terminates at specific sites in the chromosome called **termination (*ter*) sequences**. Since there are two replication forks moving in opposite directions around the circular chromosome, there are also two *ter* sequences that stop the advancement of the replication forks. One *ter* sequence is called **T1**, the other is called **T2** (see **figure 6.9**).

Proteins called **termination utilization substances (Tus)** bind to the T1 and T2 sequences. Tus proteins displace the replisomes from the two replication forks, terminating DNA replication.

Once replication ceases, **DNA ligase** forms the final covalent bond between the 5’ and 3’ ends of each daughter DNA strand, resulting in two double-stranded circular *E. coli* chromosomes.

Occasionally, the two chromosomes produced by replication are

intertwined like the links in a chain. These intertwined DNA molecules are called **catenanes**. Catenanes must be separated prior to the division of the *E. coli* cytoplasm. DNA gyrase cuts one chromosome (both DNA strands are cut), passes the other chromosome through the break, and seals the break to generate two separate chromosomes that can be distributed properly to the progeny bacterial cells.

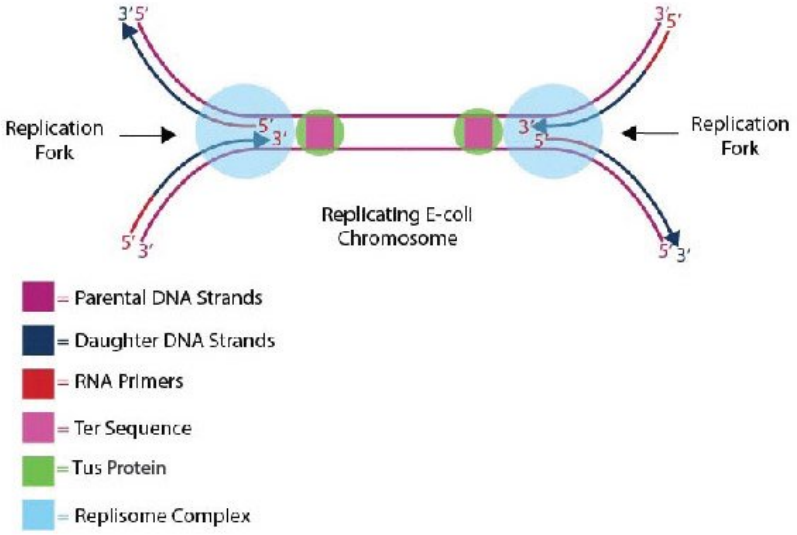


Figure 6.9 ***Termination of Replication in E. coli*** *--- Image created by KMD*

Key Questions

- What are the names of the DNA sequences that participate in replication termination in *E. coli*?
- What are the names and functions of the three proteins that participate in replication termination in *E. coli?*
- How are catenanes resolved?

B. DNA Replication in Eukaryotes

Eukaryotic Origins

Eukaryotic DNA replication is more complex than replication in prokaryotes. This is because eukaryotic genomes are generally larger than prokaryotic genomes, and the genetic material in eukaryotes is organized into linear chromosomes. However, the good news is that the replication process is similar in prokaryotes and eukaryotes and many of the DNA replication proteins (helicases, primases, and polymerases) identified in bacteria have eukaryotic counterparts that function in the same way.

One major difference between prokaryotic and eukaryotic DNA replication is that eukaryotic chromosomes have multiple replication origins (see **figure 6.10**). Like bacteria, DNA replication proceeds bidirectionally from each origin, with the formation of two replication forks per origin. As replication occurs, the replication forks from adjacent origins fuse, eventually producing two identical DNA molecules called sister chromatids.

In a model eukaryotic organism, the bread yeast *Saccharomyces cerevisiae*, the 250–400 origins are called **ARS elements.** Yeast ARS elements have the following features:

- ARS elements are approximately 50 base pairs (bp) in length.
- ARS elements are AT-rich. The presence of numerous AT base pairs in the origin promotes DNA strand separation.
- ARS elements contain an **ARS consensus sequence (ACS)**. This ARS consensus sequence is the binding site for the ORC protein complex (see below).

The DNA replicated from a single ARS element is called a **replicon**. Most eukaryotic chromosomes have multiple replicons. For example,

S. cerevisiae contains 250–400 replicons per genome, while the human genome is thought to contain approximately 25,000 replicons.

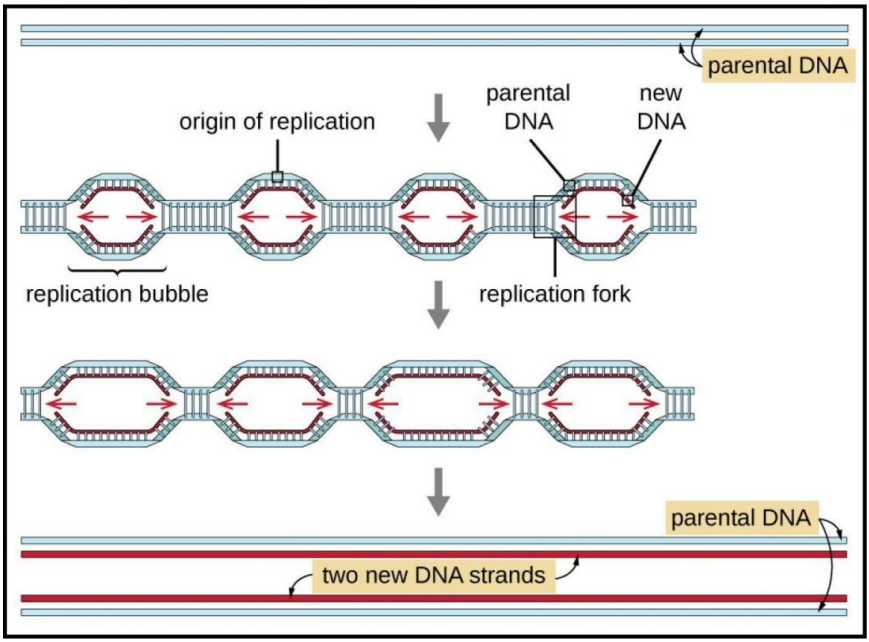


Figure 6.10 ***Eukaryotic Chromosomes Have Multiple Origins*** *--- This image is used from OpenStax (access for free at https://books.byui.edu/-vuzA)*

Key Questions

- How is replication in prokaryotes and eukaryotes similar?
- What are some differences between prokaryotic and eukaryotic replication?
- What are some of the features of a eukaryotic ARS element?

Replication Initiation in Eukaryotes

A multi-subunit protein complex called the **prereplication complex**

(preRC) assembles on ARS elements and initiates DNA replication in eukaryotes (see **figure 6.11**). The preRC contains the following protein components:

- The **origin recognition complex (ORC)**. ORC is a multi-subunit protein complex that binds directly to the ARS consensus sequence within the ARS element.
- **Regulatory proteins**. Two regulatory proteins called **cdc6** and **cdt1** bind to ORC and function to inhibit the initiation of DNA replication during the G_1, G_2, and M phases of the cell cycle. During the synthesis (S) phase of the cell cycle, cdc6 and cdt1 are phosphorylated by cellular kinases, causing cdc6, cdt1, and ORC to be released from the ARS element. DNA replication is then initiated.
- **MCM helicase.** Once replication is initiated by the release of cdc6, cdt1, and ORC, the MCM helicases catalyze the separation of the two parental DNA strands forming two replication forks. MCM helicase consumes ATP to form a replication fork.

After the DNA strands have separated, **replication protein A (RPA)** prevents the separated DNA strands from reforming hydrogen bonds. The eukaryotic DNA polymerases can then begin the elongation stage of DNA replication.

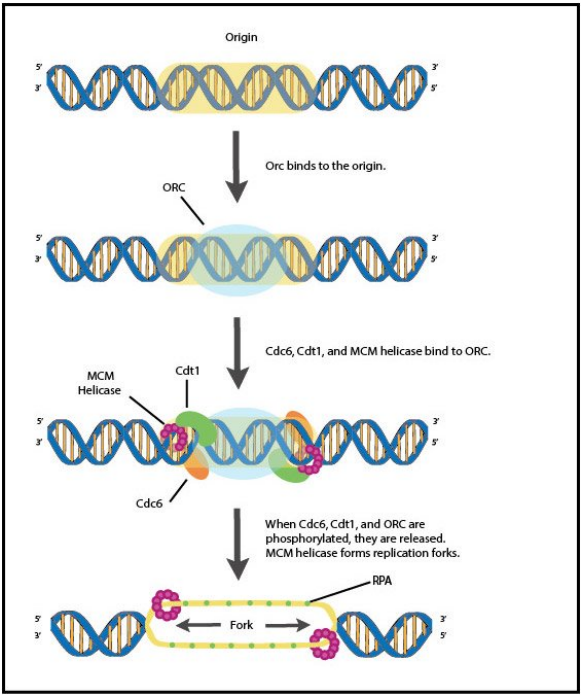


Figure 6.11 ***Replication Initiation in Eukaryotes*** *--- Image created by SL*

Key Questions

- What are the names and functions of the five proteins that participate in replication initiation in eukaryotes?

Replication Elongation in Eukaryotes

MCM helicase continues DNA strand separation during the elongation phase of DNA replication, causing the replication forks to proceed in both directions away from each origin. **RPA** prevents the separated DNA strands from reforming hydrogen bonds.

The separation of the DNA strands by MCM helicase generates positive supercoiling ahead of each replication fork. **Topoisomerase**

II is located ahead of each replication fork and produces negative supercoiling to compensate for the positive supercoiling produced by MCM helicase. Topoisomerase II consumes ATP.

There are over a dozen different DNA polymerases in a typical eukaryotic cell. These eukaryotic DNA polymerases are named according to the Greek alphabet (α, β, γ, etc.). **DNA polymerase alpha (α), DNA polymerase delta (δ)**, and **DNA polymerase epsilon (ε)** are multi-subunit enzymes involved in replicating nuclear DNA in eukaryotes (see **figure 6.12**).

DNA polymerase α associates with **DNA primase** to form a complex that synthesizes short RNA-DNA strands (10 RNA nucleotides followed by 10–30 DNA nucleotides) that are used as primers by DNA polymerases δ and ε. DNA primase synthesizes the RNA component of the primer, while DNA polymerase α synthesizes the DNA component of the primer. DNA polymerase α has both 5′ to 3′ polymerase and 3′ to 5′ exonuclease (proofreading) activity. Once the primer is made, DNA polymerase α is released and is replaced by either DNA polymerase δ or DNA polymerase ε (**polymerase switch)**.

DNA polymerases δ and ε are processive enzymes. These DNA polymerases bind to a protein called **proliferating cell nuclear antigen (PCNA)**, which clamps the DNA polymerases to the template DNA strands. DNA polymerase ε is thought to synthesize the leading strand, whereas DNA polymerase δ is thought to synthesize the lagging strand. Both DNA polymerases ε and δ contain 5′ to 3′ polymerase and 3′ to 5′ exonuclease (proofreading) activity. All three eukaryotic DNA polymerases discussed above consume dNTPs during DNA synthesis.

Flap endonuclease (**Fen1**) is a protein that removes the RNA primers from the replicating DNA, and **DNA ligase I** forms the final covalent bonds to link adjacent Okazaki fragments. DNA ligase I consumes ATP.

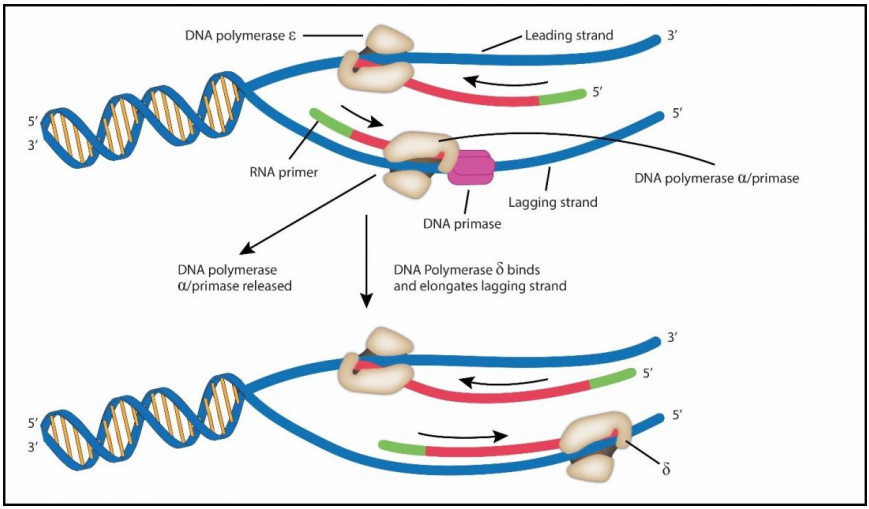


Figure 6.12 ***Replication Elongation in Eukaryotes*** *--- Image created by SL.*

Key Questions

- What are the eukaryotic equivalents of the *E. coli* enzymes DNA helicase, SSBPs, DNA gyrase, DNA primase, DNA polymerase III holoenzyme, DNA polymerase I, and DNA ligase?
- Which eukaryotic replication enzyme synthesizes the leading DNA strand?
- Which eukaryotic replication enzyme synthesizes the lagging DNA strand?
- Which eukaryotic replication elongation enzymes consume ATP?
- Which eukaryotic replication elongation enzymes consume dNTPs?

Replication at Chromosome Ends

The 3′ ends of the parental (template) DNA strands within linear eukaryotic chromosomes present a potential problem during DNA

replication. Synthesis of DNA by the eukaryotic DNA polymerases requires a 3’-OH group provided by a primer. Suppose a primer is made for the daughter DNA strand directly opposite the 3’ end of the parental DNA strand. Once this primer is used for DNA synthesis, the primer is removed with the hope that DNA replication will fill in the primer gap. However, DNA polymerases cannot fill in the primer gap at the end of the chromosome because DNA polymerases require a 3’-OH group to begin DNA synthesis. As a result, this primer gap is not filled in with DNA and the newly synthesized DNA strand is shorter than its template DNA strand. This end replication problem would result in the progressive shortening of linear DNA molecules with each round of DNA replication. Eventually, this shortening would delete genes and have a negative effect on the phenotype of the cell.

Eukaryotes solve this potential DNA replication problem by lengthening the 3’ ends of the parental DNA strands prior to DNA replication using an enzyme called **telomerase** (see **figure 6.13**). Telomerase contains both an RNA component (**TERC**) and a protein component (**TERT**); telomerase is an example of a **ribonucleoprotein**. TERC forms hydrogen bonds with the 3’ overhang DNA sequence at the ends of linear chromosomes. Once bound to the 3’ end of the DNA, TERT catalyzes the synthesis of additional telomere DNA repeat sequences at the 3’ end of the parental DNA strand using the TERC component of telomerase as a template. The synthesis of additional telomere repeats by telomerase occurs in the 5’ to 3’ direction. Because telomerase synthesizes DNA in the 5' to 3' direction and requires a 3'-OH group for DNA synthesis, telomerase is a DNA polymerase.

Once the 3’ end of the parental DNA strand is lengthened by telomerase, DNA replication of the daughter DNA strand can occur by the synthesis of a primer complementary to the repeats added by telomerase, followed by DNA synthesis using the eukaryotic DNA polymerase δ.

To sum this all up, telomerase lengthens the parental DNA strands, so that DNA replication can make the daughter DNA strands shorter. The net result is that the overall chromosome length does not change significantly after DNA replication has occurred.

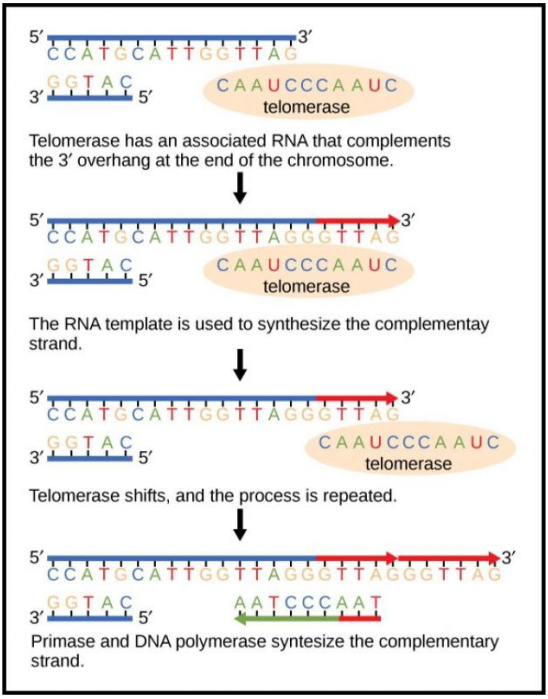


Figure 6.13 ***Telomerase Mechanism*** *--- This image is used from OpenStax (access for free at https://books.byui.edu/-vuzA)*

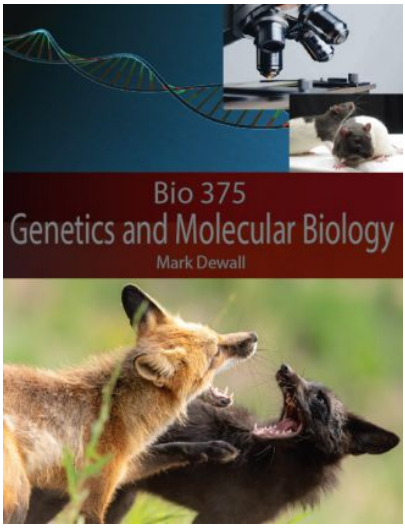
Key Questions

- Describe the end replication problem for linear chromosomes.
- How is this end replication problem solved in eukaryotes?
- What are the functions of the two components of telomerase?

Review Questions

Fill in the Blank:

1. The enzyme _____ methylates adenine to activate DNA replication in bacteria.
2. The enzyme _____ connects adjacent Okazaki fragments together during DNA replication in *E. coli*.
3. The _____ protein is the eukaryotic equivalent of SSBPs.
4. The enzyme _____ is composed of two types of subunits, called TERC and TERT.
5. During DNA replication, the template DNA strands are read by DNA polymerases in the _____ direction, while the daughter DNA strands are synthesized in the _____ direction.
6. Phosphorylation of _____ and _____ initiates DNA replication in eukaryotic organisms.
7. _____ is a eukaryotic enzyme that produces replication forks, while _____ is an *E. coli* enzyme that alleviates positive supercoiling ahead of each replication fork.
8. The _____ subunit of the DNA polymerase III holoenzyme is responsible for proofreading, while the _____ subunit is responsible for DNA synthesis.
9. _____ is an unusual DNA polymerase that contains a built-in RNA template molecule.
10. The enzyme _____ has both 5’- 3’ polymerase and 5’ - 3’ exonuclease activity.
11. _____ binds directly to the ARS element, while _____ synthesizes the leading strand in eukaryotes.



Dewall, M. (n.d.). *BIO 375: Genetics and Molecular Biology*. BYU-I Books. https://books.byui.edu/genetics_and_molecul